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# Tri- and tetraterpenoid hydrocarbons in the Messel oil shale

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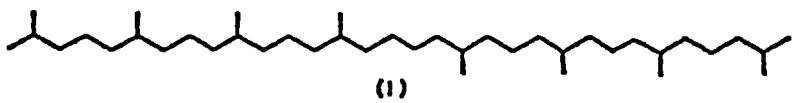
**Abstract**—The high molecular weight constituents of the branched and cyclic hydrocarbon fraction of the Messel oil shale (Eocene) have been examined by high resolution gas chromatography and combined gas chromatography–mass spectrometry. The following compounds are present: perhydrolycopene (1; lycopane), together with one or more unsaturated analogues with the same skeleton; a series of 4-methylsteranes (2c) in higher abundance than their 4-desmethyl analogues; two series of pentacyclic triterpanes, one series ($C_{27}$–$C_{32}$) based on the hopane structure (3a–e), and the other ($C_{27}$–$C_{31}$) based on the 17α-H hopane structure (3a–d, 17αH); and an intact triterpene hop-17(21)-ene [3c, Δ17(21)]. Only two additional triterpanes were detected in minor concentrations, viz. 30-normoretane (3b, 21αH) and a $C_{31}$ triterpane based on the hopane/lupane-type skeleton. The presence of these compounds suggests a significant microbial contribution to the forming sediment. Comparison of the tri- and tetraterpenoid hydrocarbons with those of the Green River Shale indicates differences in the organisms contributing to the two sediments.

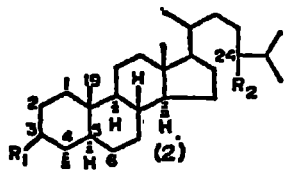
## INTRODUCTION

THE MESSEL oil shale is an organic-rich Eocene sediment (~ 50 × $10^6$ yr old) located 9 km north-east of Darmstadt, Germany. Geological studies indicate a particularly uneventful history and it is apparent that the sediment has experienced milder conditions (probably no more than 40°C) than those of the well-documented Green River Formation oil shale, U.S.A., of the same age. The shale is preserved in a basin 1000 m long and 700 m wide and the organic-rich layer has not been buried deeper than about 200 m (MATTHES, 1968). It was probably deposited in a series of shallow swampy lakes linked by slow-moving fluvial systems; analyses of fossil plants and pollens (MATTHES, 1968; SITTLER, 1968) indicate that a hot, damp, tropical climate existed at the time of deposition. In the present study the sediment samples analysed comprise mainly gyttja (sapropelic black mud) with fine sandy clay intercalations (MATTHES, 1968). The shale is composed of 25 per cent organic material, 35 per cent inorganic material and 40 per cent water (MATTHES, 1968); the inorganic portion is mainly montmorillonite, and most of the organic carbon is present as kerogen.

Previous studies of the soluble fraction have identified isoarborinol (4a) (ALBRECHT and OURISSON, 1969), arborinone (4b) and friedelin (5), a series of 4α-methylstanols (2a) and 4α-methylstanones (2b), and very small quantities of phytosterols (6a,b,c) (MATTERN *et al.*, 1970). Preliminary analysis of the hydrocarbons (ALBRECHT, 1969) indicated the absence of arborane, arborene and squalene; *n*-alkanes in the range *n*-$C_{23}$ to *n*-$C_{33}$ (C.P.I. > 6) are present, the main component being *n*-$C_{27}$. The lower molecular weight region of the branched and cyclic alkane fraction contains mainly isoprenoid alkanes with carbon numbers $C_{15}$ (farnesane),

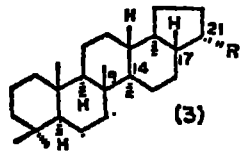


(1)



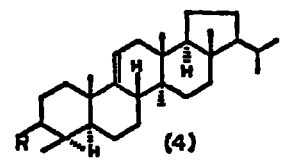
(2)

2a $R_1$ = OH, $R_2$ = H, $CH_3$, $C_2H_5$
b $R_1$ = O, $R_2$ = H, $CH_3$, $C_2H_5$
c $R_1$ = H, $R_2$ = H, $CH_3$, $C_2H_5$



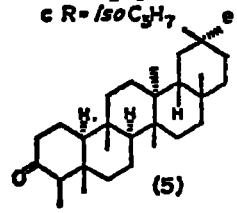
(3)

3a R = H
b R = $C_2H_5$
c R = *iso* $C_3H_7$
d R = *sec* $C_4H_9$
e R = *sec* $C_5H_{11}$

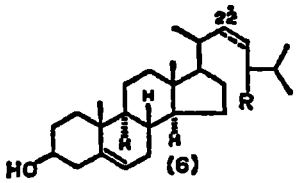


(4)

4a R = OH
b R = O

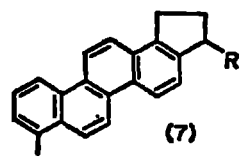


(5)



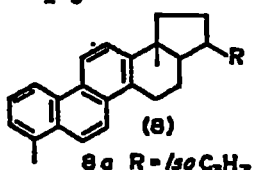
(6)

6a R = $CH_3$
b R = $C_2H_5$
c R = $C_2H_5$ and Δ-22



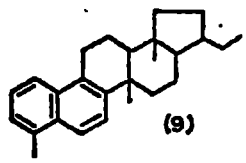
(7)

7a R = *iso* $C_3H_7$
b R = $C_2H_5$



(8)

8a R = *iso* $C_3H_7$
b R = $C_2H_5$



(9)

$C_{16}$, $C_{18}$, $C_{19}$ (pristane) and $C_{20}$ (phytane), in the relative abundances $C_{16} > C_{20} > C_{18} > C_{19} > C_{15}$ (Albrecht, 1969). Three types of aromatic hydrocarbons were isolated and mass spectrometric data indicated components of the formulae $C_{25}H_{24}$ (7a), $C_{24}H_{22}$ (7b), $C_{28}H_{30}$ (8a), $C_{25}H_{28}$ (8b) and $C_{26}H_{34}$ (9), compatible with the proposed structures indicated. The present study describes a detailed examination of the high molecular weight terpenoid components of the alkane and alkene fractions. A preliminary report of part of this work has appeared elsewhere (Ensminger *et al.*, 1972).

## Experimental

The authentic standards used were: lycopane from catalytic hydrogenation of lycopene (Hoffmann La Roche) in ethyl acetate over palladium/charcoal; 1α-, 2α-, 3β- and 4α-methylcholestanes (Kimble *et al.*, in press); hopane, 17αH-hopane, moretane, 17αH-moretane from

Prof. R. E. Corbett; 22,29,30-trisnorhopane, 30-norhopane, homohopane and 17αH analogues (Ensminger *et al.*, 1974); cholestane; stigmastane from Dr. W. McCrae.

*Gas–liquid chromatography (GLC)*

GLC was initially carried out using a 10 ft × $\frac{1}{16}$ in. stainless steel packed column (3 per cent OV-17 on 100–120 mesh Chromosorb W; 270°C; nitrogen carrier gas, 10–12 ml/min), and a Perkin–Elmer Mark 1 F-11 gas chromatograph (injector block 290–310°C).

Preparative GLC conditions for lycopane were:—column 10 ft × $\frac{1}{4}$ in., 3 per cent SE 30 on 80–100 mesh Gas Chrom Q, 60 ml/min Ar, programmed from 150–300°C; effluent trapped in liquid nitrogen. Capillary GLC was carried out on three stainless steel open tubular capillary columns as follows:—

(i) 75 ft × 0·01 in. i.d. column coated with OV-101 in a Perkin–Elmer 226 gas chromatograph incorporating a pre-column carrier gas splitter. Carrier gas (He) flow rate was 2 or 3 ml/min; the injector block was maintained at 300°C and the column was operated isothermally at 250°C. Column efficiencies were calculated to be 25,000, 20,000 and 15,000 theoretical plates for $n$-$C_{28}$, $n$-$C_{32}$ and $n$-$C_{36}$ standard alkanes, respectively.

(ii) 150 ft × 0·01 in. i.d. column coated with Dexsil 300 (Perkin–Elmer Ltd.) was used in a Perkin–Elmer Mark II F-11 gas chromatograph. The flow rate (He) was 3 ml/min; the injector block was maintained at 320°C, and the column was operated isothermally at 280°C. Column efficiency was calculated to be 59,000 theoretical plates for 5α-cholestane.

(iii) 100 ft × 0·01 in. i.d. column coated with OV-101 (Perkin–Elmer Ltd.) was used as described in (ii), except for isothermal operation at 250°C. Column efficiency was calculated to be 25,000 theoretical plates for 5α-cholestane.

*Combined gas chromatography–mass spectrometry (GC–MS)*

Mass spectra were obtained using a Varian Aerograph 1200 gas chromatograph coupled by an all-glass single-stage Watson–Biemann He separator to a Varian MAT CH-7 single focussing mass spectrometer. The accelerating potential was 70 eV, the filament current 100 or 300 μA, and the instrument resolution 800–1000. The gas chromatograph was used initially with the packed column described above and subsequently with the capillary columns described in (ii) and (iii) above. Under these conditions the efficiency of column (ii) was *ca.* 55,000 theoretical plates for 5α-cholestane.

*Isolation and analysis of branched and cyclic alkanes and alkenes*

Analyses of the alkanes were carried out on two different solvent extracts of the shale.

(i) Extract I was obtained using a mixture of light petroleum (b.p. 60–80°C) and ethyl acetate (4:1) and the total hydrocarbons (0·25 per cent of 1 kg of dry rock) were isolated by column chromatography ($SiO_2$/light petroleum, b.p. 60–80°C). Additional column chromatography ($SiO_2$/light petroleum) and thin layer chromatography using silica gel impregnated with 10 per cent $AgNO_3$ ($Ag^+$—TLC) gave the total alkanes (226 ppm). Treatment of the total alkanes with 5 Å molecular sieve (O'Connor *et al.*, 1962), and urea adduction (Cox, 1971) gave the total branched and cyclic alkanes (56 ppm) which were re-purified by $Ag^+$—TLC (hexane) and further simplified by thiourea adduction (Murphy *et al.*, 1967). The resulting adduct and non-adduct fractions were analysed by packed column and capillary column (i) GLC and packed column GC–MS.

(ii) Extract II was obtained using cyclohexane; elemental sulphur was removed by the method of Blumer (1957). The sulphur-free extract (0·2 per cent of 1·5 kg of rock) was chromatographed (neutral alumina column/hexane) to give the total hydrocarbons (0·04 per cent) from which the branched and cyclic hydrocarbons were isolated on adduction of the straight chain components with urea. The alkanes (*ca.* 20 ppm) and alkenes (*ca.* 270 ppm) were separated by $Ag^+$—TLC (hexane developer).

An aliquot of the branched and cyclic alkenes from Extract II was hydrogenated ($PtO_2$/C EtOAc/atmospheric pressure of $H_2$) and the resulting alkanes were isolated by $Ag^+$—TLC.

The alkane and alkene fractions obtained (Extract II) were analysed by capillary column [described under (ii) and (iii) above] GLC and by packed column and capillary GC–MS (columns ii and iii).

## RESULTS

Identifications were based on high resolution gas chromatography on Dexsil 300 and OV-101 by coinjection with authentic standards and comparison of mass spectra with those of the standards. This method is similar to that used by HENDERSON *et al.* (1968a, b) for the identification of steranes and triterpanes in the Green River Formation oil shale. The chromatogram of the urea alkane non-adduct (Extract II) on Dexsil 300 is shown (Fig. 1), the peak numbers corresponding to the components summarized in Table 1.

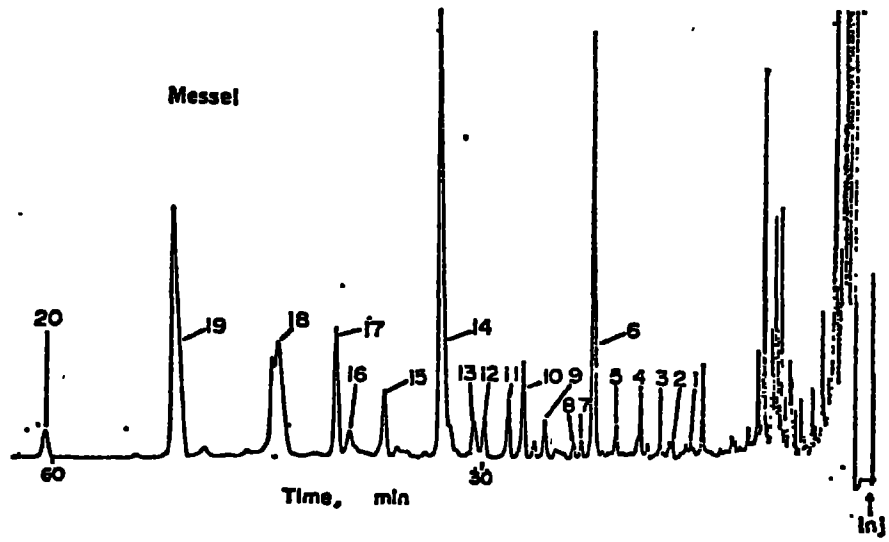



Fig. 1. Capillary gas chromatogram of Messel branched and cyclic alkanes (Extract II, urea non-adduct) on Dexsil 300 (isothermal 280°C).

### *Lycopane (perhydrolycopene, 1)*

The mass spectrum (Fig. 2a) of the main component in the thiourea adduct fraction of Extract I (also peak 18 in Fig. 1) indicated an acyclic alkane with extensive branching. Detailed information could not be obtained from the GC–MS spectrum of the region above m/e 480 as the ions were of particularly low intensity. The GC–MS spectrum (Fig. 2b) of an authentic sample of lycopane from catalytic hydrogenation of lycopene strikingly resembled that of the Messel component but again it was not possible to determine the molecular ion exactly. More intense spectra of this region (incorporated into Figs. 2a, b) were obtained by direct insertion mass spectrometry (perfluorokerosene marker) of the Messel component isolated by preparative GLC and of authentic lycopane.

Further proof of identity was obtained by capillary column GLC. Coinjection of authentic lycopane (1; Kovats indices: 3471 on Dexsil 300, 3506 on OV-101) with the thiourea adduct fraction of Extract I and with the total branched and cyclic alkanes from Extract II (Fig. 1), produced peak enhancement. The similarities in

Table 1. Polyterpenoid branched and cyclic alkanes identified in Messel oil shale by capillary GC–MS
(Peak numbers refer to Fig. 1)

| Peak | Formula | Assignment | Structure |
|---|---|---|---|
| 1 | $C_{27}H_{48}$ | 5β-Cholestane | 10a; 5βH |
| 2 | $C_{27}H_{48}$ | Cholestane | 10a |
| 3 | $C_{28}H_{50}$ | 4-Methyl-5β-cholestane | 2c; 5βH; $R_2 = H$ |
| 4 | $C_{28}H_{50}$ | 4-Methylcholestane | 2c; $R_2 = H$ |
| 5 | $C_{27}H_{46}$ | 17αH-trisnorhopane | 3a; 17αH |
| 6 | $C_{27}H_{46}$ | Trisnorhopane | 3a |
| 7 | $C_{29}H_{52}$ | 4-Methylergostane | 2c; $R_2 = CH_3$ |
| 8 | $C_{29}H_{52}$ | Stigmastane | 10c |
| 9 | $C_{29}H_{50}$ | 17αH-Norhopane | 3b; 17αH |
| 10 | $C_{30}H_{54}$ | 4-Methylstigmastane | 2c; $R_2 = C_2H_5$ |
| 11 | $C_{29}H_{50}$ | 30-Normoretane | 3b; R = 21αH |
| 12 | $C_{30}H_{52}$ | 17αH-Hopane | 3c; 17αH |
| 13 | $C_{30}H_{50}$ | Pentacyclic triterpene | |
| 14 | $C_{29}H_{50}$ | Norhopane | 3b |
| 15 | $C_{31}H_{54}$ | 17αH-Homohopane | 3d; 17αH |
| 16 | $C_{31}H_{54}$ | Pentacyclic triterpane | |
| 17 | $C_{30}H_{52}$ | Hopane | 3c |
| 18 | $C_{40}H_{82}$ | Lycopane | 1 |
| 19 | $C_{31}H_{54}$ | Homohopane | 3d |
| 20 | $C_{32}H_{56}$ | Bishomohopane | 3e |

the GC–MS spectrum of peak 18 (Fig. 1) and in the direct insertion spectra of authentic lycopane and the preparative GLC sample which included the shoulder on peak 18 suggest that the shoulder may be a compound similar to lycopane; indeed, its presence could indicate separation of diastereoisomers of lycopane.

In both spectra (Fig. 2) the ion at m/e 560 could represent the molecular ion for a mono-unsaturated or monocyclic alkane impurity of formula $C_{40}H_{80}$. However, this is unlikely because neither spectrum contains fragment ions (e.g. M-15 and

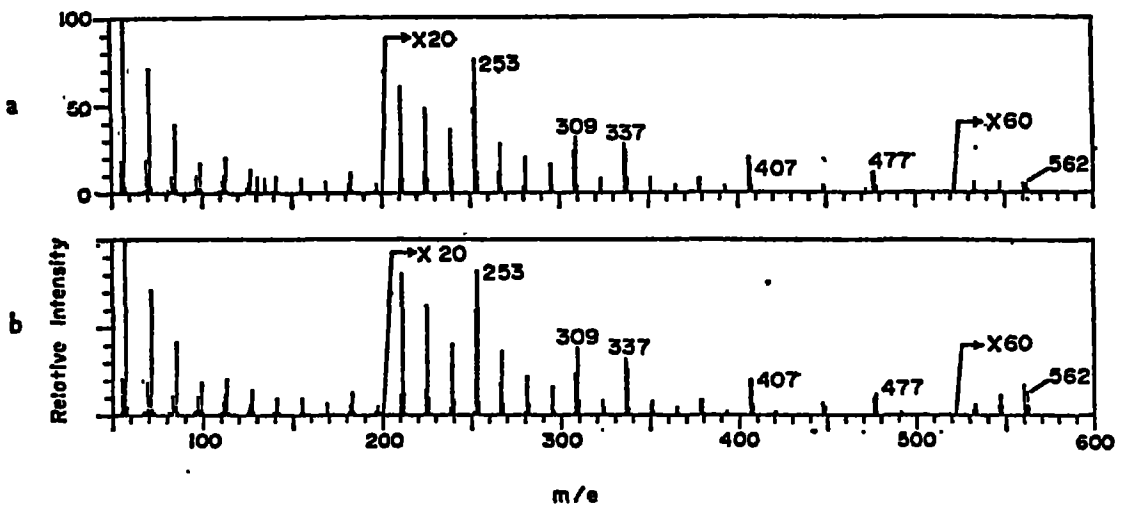



Fig. 2. Mass spectra from capillary GC–MS on Doxsil 300 of (a) Messel component 18 in Fig. 1 and (b) authentic lycopane ($C_{40}H_{82}$, 1), respectively.

$C_nH_{2n-1}$) expected from a monocyclic carotenoid alkane such as perhydro-$\gamma$-carotene. Also, bromination of authentic lycopane followed by $Ag^+$-TLC purification failed to produce any change in the spectrum. The M-2 ion at m/e 560 therefore appears to be a genuine fragment ion, as do those at m/e 252, 308, 336, 406 and 476, of the $C_nH_{2n}$ series.

*Methylsteranes and steranes* (*Table* 1)

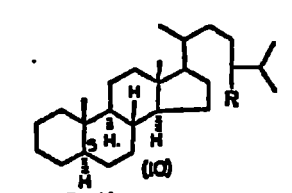
The spectra of four cycloalkanes ($C_{28}H_{50}$, $C_{29}H_{50}$, $C_{29}H_{52}$ and $C_{30}H_{54}$) present in the thiorea adduct (Extract I) and in the urea non-adduct (Extract II, Peaks 3, 4, 7 and 10, Fig. 1) showed fragmentation patterns analogous to those of the authentic sterane series cholestane ($C_{27}H_{48}$, 10a), ergostane ($C_{28}H_{50}$, 10b) and stigmastane ($C_{29}H_{52}$, 10c) (HENDERSON *et al.*, 1968a, b; ANDERSON *et al.*, 1969). The major features of the seven spectra are summarized in Table 2. Thus, for the Messel

Table 2. Structurally significant ions in the mass spectra of four Messel oil shale alkanes and those of authentic (ANDERSON *et al.*, 1969) cholestane, ergostane and stigmastane

| Peak no. (Fig. 1) | Formula | Assignment | Structure | Significant ions | M-124 | M-15 | M |
|---|---|---|---|---|---|---|---|
| 3 | $C_{28}H_{50}$ | 4-Methyl-5$\beta$-cholestane | 2c; 5$\beta$H; $R_2$ = H | 163/165 217 231 232 246 | 262 | 371 | 386 |
| 4 | $C_{28}H_{50}$ | 4-Methylcholestane | 2c; $R_2$ = H | 163 217 231 232 246 | 262 | 371 | 386 |
| 7 | $C_{29}H_{52}$ | 4-Methylergostane | 2c; $R_2$ = $CH_3$ | 163 217 231 232 246 | 276 | 385 | 400 |
| 10 | $C_{30}H_{54}$ | 4-Methylstigmastane | 2c; $R_2$ = $C_2H_5$ | 163 217 231 232 246 | 290 | 399 | 414 |
| | | | | | M-110 | M-15 | M |
| — | $C_{27}H_{48}$ | Cholestane | 10a | 149 203 217 218 232 | 262 | 357 | 372 |
| — | $C_{28}H_{50}$ | Ergostane | 10b | 149 203 217 218 232 | 276 | 371 | 386 |
| — | $C_{29}H_{52}$ | Stigmastane | 10c | 149 203 217 218 232 | 290 | 385 | 400 |

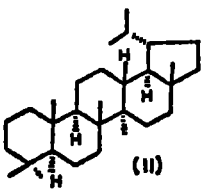
components the ions from ring D fragmentation occur at m/e 231 and 232 (cf. m/e 217 and 218 for the $C_{27}$–$C_{29}$ steranes) and the fragment ions at m/e 163, 217 and 246 have similar relative intensities to the ions at m/e 149, 203 and 232, respectively, in the $C_{27}$–$C_{29}$ steranes whose spectra have been previously reported in detail (HENDERSON *et al.*, 1968a, b; ANDERSON *et al.*, 1969). These features indicate that the Messel components are methyl-substituted analogues of cholestane, ergostane and stigmastane, the position of substitution being limited to one of the carbon atoms 1, 2, 3, 4, 6 and 19 (TOKÉS *et al.*, 1968). An authentic sample (KIMBLE *et al.*, in press) of 4$\alpha$-methylcholestane (2c, $R_2$ = H) gave the expected spectrum and enhanced peak 4 (Fig. 1) when coinjected on Dexsil 300. Synthetic samples of 1$\alpha$-methyl-, 2$\alpha$-methyl-, 3$\beta$-methyl-, and 4$\alpha$-methylcholestane which have similar mass spectra were all separable by capillary column GLC on Dexsil 300 (KIMBLE *et al.*, in press). The evidence suggests that peaks 3, 7 and 10 (Fig. 1) are also 4-methylsteranes. Comparison of the intensities of the ions at m/e 163 and 165 in the spectrum of peak 3 with those in the spectrum of the assigned 4-methylcholestane suggest that peak 3 is the 5$\beta$ analogue of 4-methylcholestane, the relative intensities of the two ions paralleling those of the ions at m/e 149 and 151 in the spectra of cholestane and 5$\beta$-cholestane (GALLEGOS, 1971). Peaks 1, 2 and 8 were assigned in the usual way

as 5$\beta$-cholestane (10a; 5$\beta$H), cholestane (10a) and stigmastane (10c) respectively, by, comparison with authentic standards.

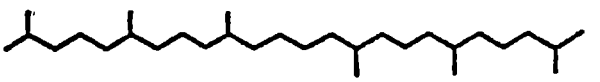


(10)

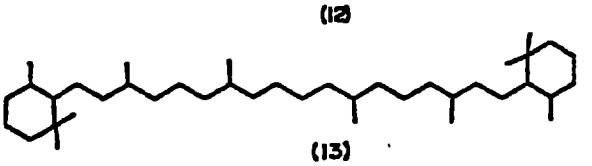
a R = H
b R = $CH_3$
c R = $C_2H_5$



(11)



(12)



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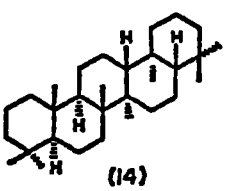

(14)

The stereochemistry of the C-4 methyl group remains unproven, as the 4$\alpha$- and 4$\beta$-isomers may not be distinguishable under the GLC conditions used. Considerations of sterol biosynthesis and the relative abundance of the 4$\alpha$-methylsterols compared to 4$\beta$-methylsterols in organisms (Rees and Goodwin, 1972, and references therein), together with the inherent stability of the 4$\alpha$-isomers with respect to the 4$\beta$-methyl compounds (Pyrek, 1969), suggest that these geological methyl steranes are probably the 4$\alpha$-methyl series.

*Triterpanes** (*Table* 1)

The spectra of the major higher molecular weight components (listed in Tables 1 and 3) in the thiourea non-adduct fraction of Extract I all showed a very intense ion at m/e 191, typically found in pentacyclic triterpanes with two adjacent quaternary carbon atoms at C-8 and C-14 [for example, structures (3a–e)] or at C-8 and C-13 (Kimble *et al.*, in press). The molecular formulae (Table 3) range from $C_{27}H_{46}$ to $C_{32}H_{56}$ with the notable absence of $C_{28}H_{48}$; this range is related to the nature of the E ring substituent R which is also reflected in the masses of the intense ions at m/e 149, 177, 191, 205 and 219 (3a–e).

* In the present context, 'triterpane' is used to imply the saturated hydrocarbons presumably derived from triterpenoids, and to include their proximate higher ($C_{31}$, $C_{32}$ . . .) and lower ($C_{29}$, $C_{28}$, $C_{27}$) homologues.

Table 3. $C_{27}$–$C_{32}$ pentacyclic triterpanes in the Messel and Green River Shales

| Formula | Messel triterpanes | Green River triterpanes* |
|---|---|---|
| $C_{27}H_{46}$ | Trisnorhopane (3a, *ca.* 3·5%)†<br>17α(H)-Trisnorhopane (3a, 17α(H), <1%) | $C_{27}H_{46}$ (trace) |
| $C_{28}H_{48}$ | n.d. | $C_{28}H_{48}$ (trace) |
| $C_{29}H_{50}$ | Norhopane (3b, *ca.* 3·5%)<br>17α(H)-Norhopane (3b, 17α(H), <1%)<br>Isoadiantane (3b, 21α(H), <1%) | 17α(H)-norhopane (3b, 17α(H), *ca.* 2%) |
| $C_{30}H_{52}$ | Hopane (3c, *ca.* 2%)<br>17α(H)-Hopane (3c, 17α(H), <1%) | 17α(H)-hopane (3c, 17α(H), *ca.* 9%‡)<br>gammacerane (14, *ca.* 3%)<br>$C_{30}H_{52}$ (*ca.* 4%)<br>$C_{30}H_{52}$ (<1%) |
| $C_{31}H_{54}$ | Homohopane (3d, *ca.* 7%)<br>17α(H)-Homohopane (3d, 17α(H), <1%)<br>$C_{31}H_{54}$ (Hopane/lupane type <1%) | 17α(H)-homohopane (3d, 17α(H), <1%)<br>$C_{31}H_{54}$ (<1%) |
| $C_{32}H_{56}$ | Bishomohopane 3c (<1%) | $C_{32}H_{56}$ (<1%) |

* Taken from data of BURLINGAME *et al.* (1965); HILLS *et al.* (1966); HENDERSON *et al.* (1968a, b); GALLEGOS (1971); ANDERS and ROBINSON (1971); BALOGH *et al.* (1973); VAN DORSSELAER *et al.* (in press).

† % of branched cyclic alkane fraction.

‡ Previously tentatively identified as hopane by HENDERSON *et al.* (1968b); shown to be 17α(H) isomer by WHITEHEAD (1971) and BALOGH *et al.* (1973).

n.d. not detected.

The major $C_{31}$ triterpane (Fig. 1, peak 19) was identified (ENSMINGER *et al.*, 1972) as *one* of the two C-22 stereoisomers of homohopane (3d, R = *sec*-$C_4H_9$). In the present study the following authentic standards were coinjected with the branched and cyclic alkane fraction (Extract II, Fig. 1) on both Dexsil 300 and OV-101 phases: 22,29,30-trisnorhopane (3a); 30-norhopane (adiantane, 3b); hopane (3c); 22,29,30-trisnor-17α(H)-hopane (3a, 17α(H)); 30-nor-17α(H)-hopane (3b, 17α(H)); 17α(H)-hopane (3c); 17α(H)-homohopane (3d; 17α(H)); and 30-normoretane (isoadiantane 3b, 21α(H)). The resulting peak enhancements and the comparison of the capillary GC–MS mass spectra (Dexsil 300 and OV-101) of the standards with those of the Messel components (Fig. 3), allowed identification of components 6, 14 and 17 (Fig. 1) as the $C_{27}$, $C_{29}$ and $C_{30}$ members of the hopane series (3a–c, respectively), components 5, 9, 12 and 15 as the $C_{27}$, $C_{29}$, $C_{30}$ and $C_{31}$ members of the 17α(H)-hopane series (3a–d, 17α(H), respectively). Similarly, component 11 (Fig. 1) was identified as 30-normoretane. The assignments are summarized in Table 1. Coinjection with authentic moretane (3c, 21α(H)), 17α(H)-moretane (3c, 21α(H), 17α(H)) and arborane (cycloalkane from 4) showed that these compounds were either absent or present in very low abundances.

The Messel triterpanes, therefore, appear to form two related series, each series characterized by the stereochemistry at the D–E ring junction (i.e. carbon atom 17) and based on variations (II, $C_2H_5$, etc.) of the substituent R in the E ring. Since the

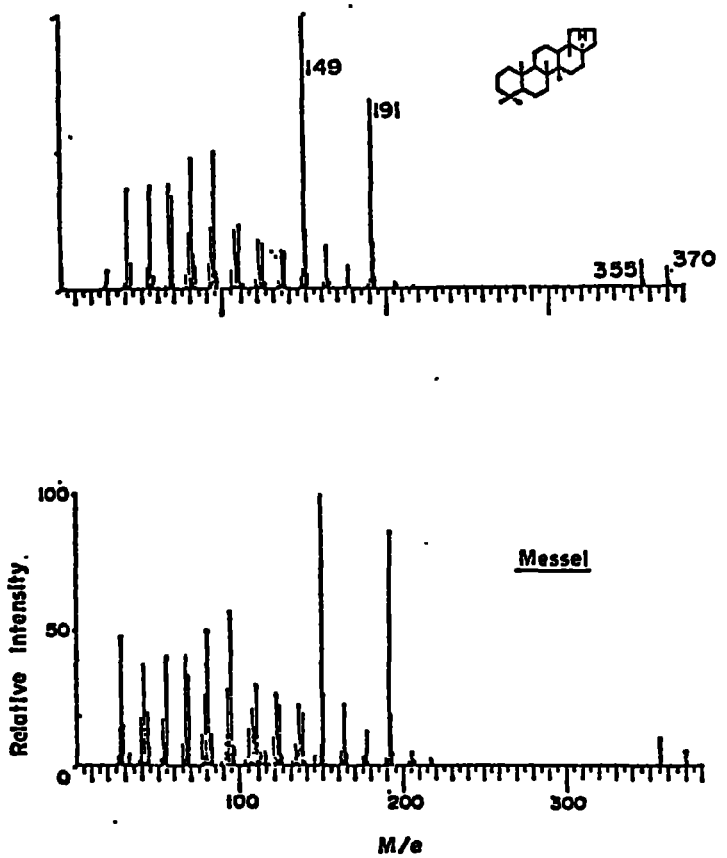


Fig. 3. Mass spectra from capillary GC–MS on Dexsil 300 of Messel component 6 in Fig. 1 and authentic 22, 29, 30-trisnorhopane (3a).

more abundant series by far is the hopane series (3a–d), it is inferred that the only $C_{32}$ triterpane present, component 20 (Fig. 1, Table 1), is the $C_{32}$ member of this series. Comparison of the mass spectrum of this component with those of the lower members of the hopane series is also in agreement with this assignment. Component 13 (Fig. 1) appears to be an unsaturated pentacyclic triterpene hydrocarbon, $C_{30}H_{50}$ (Table 1), with a spectrum corresponding to that of hopane (3) or lupane (11)-types of skeleton, i.e. a 5-membered E-ring; its presence in the branched/cyclic alkane fraction indicates that the double bond is in a hindered position. Component 16, the third $C_{31}$ pentacyclic triterpane, also appears to be related to the hopane (3) or lupane (11) skeletons but its spectrum differs from that of homohopane (3d) and 17αH-homohopane (3d, 17αH) in the relative abundances of the ions at m/e 191 and 205.

*Branched and cyclic alkenes*

Preliminary information was obtained by catalytic hydrogenation of an aliquot followed by preparative $Ag^+$–TLC. An unsaturated fraction was obtained from the products and was dominated by a single component in the higher molecular weight region. The mass spectrum indicated a molecular formula $C_{30}H_{50}$, and was similar

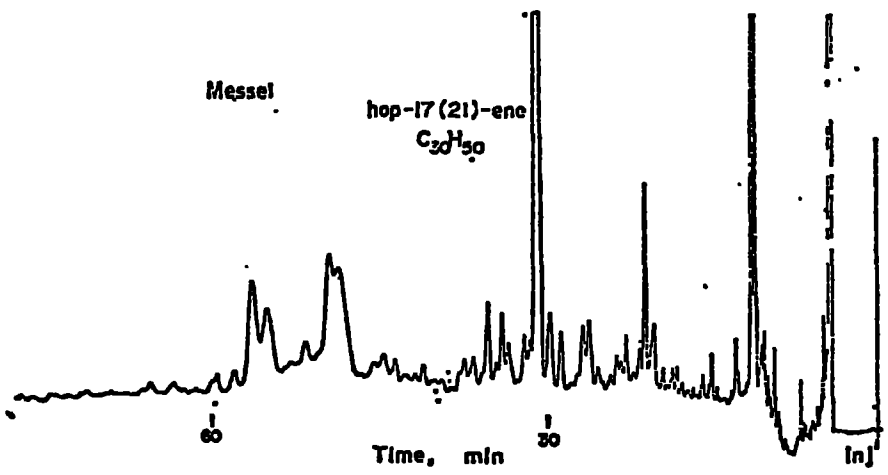


Fig. 4. Capillary gas chromatogram of Messel branched and cyclic alkenes (Extract II, urea non-adduct) on Dexsil 300 (isothermal 280°C).

to that obtained from an authentic sample of hop-17(21)-ene [3c, Δ17(21)]. This identification was confirmed by coinjection on both phases; furthermore, direct analysis of the branched and cyclic alkene fraction by GC–MS and GLC coinjection showed that the main component in Fig. 4 was hop-17(21)-ene, present intact in the shale. This component has a different mass spectrum from that of peak 13 in the branched/cyclic alkane fraction (Fig. 1, Table 1) which appears to be another hopene from its mass spectrum with a hindered double bond, since it is present in the alkanes after $Ag^+$–TLC.

A fraction, corresponding to alkanes in $Ag^+$–TLC $R_f$, was also isolated from the hydrogenation products. GLC of this fraction indicated four main components. The mass spectrum of the least volatile of these strongly resembled that obtained from authentic lycopane (Fig. 2), which produced peak enhancement on both phases. The identification of lycopane in this fraction indicates that one or more unsaturated compounds having the same carbon skeleton are present in the sediment.

## Discussion

### *Lycopane*

The identification of lycopane (1) is the first reported occurrence in a geological material, although the structurally-related $C_{30}$ compound squalane (12) has been identified in a Nigerian crude oil (Gardner and Whitehead, 1972). The bicyclic tetraterpane perhydro-β-carotene (13) has been identified in the Green River Formation oil shale (Murphy *et al.*, 1967), and mass spectral data have also indicated the presence of another $C_{40}H_{82}$ component, components of molecular formulae $C_{40}H_{82}$ to $C_{40}H_{70}$ (Gallegos, 1971) and a monocyclic $C_{40}H_{80}$ alkane (Anders and Robinson, 1971). Similar data obtained from hydrocarbon samples isolated from several deep sea (JOIDES) cores indicated $C_{40}$ compounds, including one of formula $C_{40}H_{76}$ (Simoneit and Burlingame, 1971).

Analysis of another deep sea (JOIDES) core (Hare and Hoering, 1972) indicated the presence of an acyclic $C_{40}$ monohydroxy compound, apparently having the

biosynthetically-interesting *head*-to-*head* coupling of two regular $C_{20}$ units.

The presence of tetraterpanes in the Messel and Green River Formation oil shales indicates firstly a significant contribution of carotenoids to the original sediments, and secondly that conditions in these sediments permitted the reduction of carbon–carbon double bonds and the preservation of carotenoid carbon skeletons.

In contrast to the algal origin proposed for much of the organic matter in the Green River Formation oil shale (BRADLEY, 1970), the explanation of significant quantities of lycopane and the apparent absence of perhydro-$\beta$-carotene in the Messel shale is less obvious. Acyclic carotenoids have so far only been found as *major* carotenoids in photosynthetic bacteria (GOODWIN, 1965, 1966, 1970; JENSEN, 1963, 1967; WEEDON, 1965) and the distribution of cyclic and acyclic structures within the families indicates that non-sulphur purple bacteria (anaerobic heterotrophs found in soils, stagnant ponds, etc.; VAN NIEL, 1963) are likely to be the main producers of acyclic carotenoids to the exclusion of their cyclic analogues.

*Methylsteranes*

The series of 4-methylsteranes is of particular significance in view of the co-occurrence of their corresponding saturated 3-ketones and 3-alcohols (MATTERN *et al.*, 1970) and because cycloalkanes having these carbon skeletons have not so far been reported to occur in other geological materials.

4-Monomethylsterols having $C_{28}$, $C_{29}$ or $C_{30}$ carbon skeletons exist as *minor* sterol components in a variety of plants but relatively large amounts of unsaturated 3-hydroxy derivatives of 4$\alpha$-methylcholestane have been reported in the bacterium *Methylococcus capsulatus* (BIRD *et al.*, 1971a, b).

The identification of 4-methylsteranes and their derivatives in a geological material is of interest, as the occurrence of large amounts of 4-methyl compounds relative to 4-desmethyl compounds contrasts with the relative abundances in present-day plant species. This may be explained by supposing that the original Messel flora was dominated by species synthesizing predominantly the 4-methyl structures, or that the 4-methyl compounds have survived diagenesis better than the 4-desmethyl-sterols, or that the common 4-desmethyl-phytosterols were initially incorporated into the sediment and were subsequently methylated at the 4-position. The established intermediacy of 4-methylsterols in the biosynthesis of 4-desmethyl-phytosterols (REES and GOODWIN, 1972, and references therein) supports the first explanation and further studies may identify other bacterial species that produce predominantly 4-methylsterols.

The co-occurrence of these skeletal types as alkanes, ketones and alcohols indicates that the diagenetic and/or maturation processes involved redox reactions producing ketones and hydrocarbons from biologically-occurring alcohols.

*Triterpanes*

The most abundant triterpanes present comprise a series (3a–e) based on the hopane ring structure. The presence of isoarborinol (4a) and arborinone (4b) as major constituents of the Messel shale, taken with the absence of the corresponding cycloalkane, arborane, in the hydrocarbon fraction, suggests that the geological hopane-type hydrocarbons derive from 3-desoxy-triterpanes with the same skeleton, rather than from 3-oxygenated precursors. Such 3-desoxycarbon skeletons occur in

ferns (BERTI and BOTTARI, 1968; BOTTARI *et al.*, 1972). Also, recent studies have shown that hopane (3c), hop-22(29)-ene (3c, R-*iso* $C_3H_8$) and hop-17(21)-ene [3c, Δ17(21)] are present in the bacterium *Bacillus acidocaldarius*, along with two minor components ascribed to a $C_{31}$ hopane-type alkane and alkene, respectively (DE ROSA *et al.*, 1971, 1973); hop-22(29)-ene has also been isolated from the bacterium *Methylococcus capsulatus* and other prokaryotes (BIRD *et al.*, 1971a, b). Re-examination of the mass spectrum of a polycyclic hydrocarbon isolated from each of three species of blue-green algae, *Lyngbya aestuarii*, *Nostoc* sp., and *Chroococcus turgidus* (GELPI *et al.*, 1970) suggests that this compound is probably also hop-22(29)-ene.

The second, minor triterpane series (3a–d, 17αH) present in the Messel shale has the 17αH stereochemistry; at present there is no report of the occurrence in living organisms of any compound with a carbon skeleton of this type.

Extended ($>C_{30}$) and degraded ($<C_{30}$) pentacyclic triterpanes have been detected by mass spectrometry in a variety of geological materials: oil shales (HENDERSON *et al.*, 1968a, b; DOUGLAS *et al.*, 1969; GALLEGOS, 1971; WSZOLEK *et al.*, 1971; ANDERS and ROBINSON, 1971; ARPINO *et al.*, 1971; VAN DORSSELAER *et al.*, in press), coal and lignite (MAXWELL, 1967; VAN DORSSELAER *et al.*, in press, and crude oil (DANIELLI *et al.*, 1968; HILLS *et al.*, 1970; WHITEHEAD, 1971; VAN DORSSELAER *et al.*, in press).

Such triterpane carbon skeletons may be considered to be of essentially a geochemical origin, i.e. formed by the alteration of $C_{30}$ biolipids by the physical and chemical effects of the geological environment, a biogeochemical origin, i.e. formed by the action of organisms in the forming sediment, or a biochemical origin, i.e. formed by organisms before sedimentation.

(i) *Geochemical origin of triterpanes.* The formation of degraded triterpanes containing 27 and 29, but *not* 28, carbon atoms from a $C_{30}$ precursor, is a problem similar to that of the formation of $C_{16}$, $C_{18}$ and $C_{19}$, but *not* the $C_{17}$, isoprenoid alkanes from a $C_{20}$ precursor. The $C_{28}$ compound (cf. the $C_{17}$ isoprenoid) can only be derived by cleavage of two C—C bonds located at the same carbon atom; only single carbon–carbon bond cleavages would be required for the formation of the $C_{27}$ (cf. the $C_{16}$ isoprenoid) and the $C_{29}$ (cf. the $C_{18}$ isoprenoid) structures. Participation of the insoluble organic kerogen matrix in the formation of these triterpanes has been proposed (ARPINO *et al.*, 1971). However, the subsequent identification (ENSMINGER *et al.*, 1972) of only *one* of the two C-22 stereoisomers of the $C_{31}$ triterpane homohopane in the Messel shale is inconsistent with the particular route proposed by ARPINO *et al.* (1971). In addition, the occurrence of homohopane (3d) and other alkylated and dealkylated hopane-type hydrocarbons in recently-deposited lacustrine sediments (CRANWELL, 1973; EGLINTON *et al.*, in press) indicates that such compounds can be formed at the very earliest stages of diagenesis, although it is not yet certain that the compounds in these sediments are not derived from older geological materials. However, if they are syngenetic with the contemporary sediments in which they occur, then it appears that thermal cracking (carbon–carbon bond cleavage) of the kerogen matrix is not a necessary prerequisite for their formation.

(ii) *Biogeochemical/biochemical origin of triterpanes.* Several related possibilities exist:—

(a) The alkylated, degraded and $C_{30}$ triterpane skeletons were produced by species

of the original Messel biota which were capable of synthesizing these skeletons directly (biogenesis), presumably from squalene or squalene epoxide followed by the gain or loss of carbon atoms. The occurrence of a $C_{31}$ hopane-type alkene and alkane in a bacterium (DE ROSA *et al.*, 1971, 1973) indicates that a biosynthetic pathway exists in microorganisms for the formation of the $C_{31}$ skeleton and its saturation.

(b) The triterpenoids originally deposited in the sediment were of the types found in present-day organisms (the $C_{30}$ hopane and $C_{29}$ adiantane skeletons) and carbon atoms were subsequently added and removed by microorganisms during early-stage diagenesis (bioalteration).

In cases (a) and (b), the additional carbon atoms would presumably be supplied by methionine. Mechanisms paralleling those involved in the biosynthesis of the C-24 alkylated sterols (LEDERER, 1969) can be envisaged which would be expected to form the observed single $C_{32}$ stereoisomer (R or S; ENSMINGER *et al.*, 1972) of the $C_{31}$ homohopane bearing a *sec*-butyl (1-methylpropyl) substituent.

Parallels for the specific removal of carbon atoms to form the $C_{27}$ and $C_{29}$, but not the $C_{28}$, pentacyclic structures are less obvious. The biosynthetic route for the formation of adiantane ($C_{29}$) derivatives in plants is not known, but the loss of carbon atoms from the C-4 and C-14 positions in sterols and certain tetracyclic triterpenoids proceeds by the sequential oxidation of a methyl group to a carboxyl group followed by decarboxylation (GOAD, 1969). Similar decarboxylation of a hopane skeleton would imply the stepwise removal of carbon atoms, which does not preclude formation of a $C_{28}$ carbon skeleton. It appears, therefore, that, if route (b) is the one involved, the mechanism involved must be sensitive to the precise branched structure of the isopropyl side chain and specific reactive sites in the form of functional groups must be available at the 22–29 ($C_{29}$ structure) and 21–22 ($C_{27}$ structure) bonds. Both the biologically-occurring triterpenoids 22-hydroxyhopane and hop-22(29)-ene could provide such sites, the former by dehydration [to hop-22(29)-ene and hop-21(22)-ene] and the latter by reacting directly and by double bond isomerization to hop-21(22)-ene (which occurs very readily in the laboratory with mild acids).

(c) The triterpanes could be the degradation products of a biologically-produced $C_{35}$ pentacyclic isoprenoid (WHITEHEAD, 1972). For example, the major Messel triterpane, homohopane (3d) could have arisen from decarboxylation of one of the C-22 stereoisomers of the homologous $C_{32}$ acid (3d, R = $C_3H_6CO_2H$), itself formed by degradation of a pentacyclic $C_{35}$ compound ENSMINGER *et al.*, in press). Possible precursor compounds have recently been isolated from a bacterium (FÖRSTER *et al.*, 1973).

Our present data do not allow distinction between possibilities (a), (b) and (c) but the evidence available points towards the involvement of microorganisms in the formulation of the extended and degraded hopanes in the Messel shale.

The origin of the 17$\alpha$H-configuration in the minor Messel triterpane series (3a–d, 17$\alpha$H) is also of interest because no carbon skeletons of this type have been found in living organisms. ENSMINGER *et al.* (in press) have provided evidence that the ratio of the 17$\alpha$H alkane to the 17$\beta$H alkane in geological samples provides an indication of the extent of maturation. Thus, mature sediments and crude oils are characterized

by a high abundance of the more stable 17$\alpha$H alkanes relative to the 17$\beta$H alkanes, possibly as a result of epimerization of the 17$\beta$H alkanes. This may not be the situation with respect to all samples, however, because abundant hopanes with the 17$\alpha$H configuration appear to be present in several recently-deposited lacustrine sediments (EGLINTON *et al.*, in press), suggesting that the 17$\alpha$H configuration can be formed in some samples at the earliest stages of diagenesis, although their syngenetic origin with these contemporary sediments is not yet fully proved.

## CONCLUSIONS

The carbon skeletons of the tri-and tetraterpenoid alkanes of the Messel shale provide an interesting comparison with those of the well-studied Green River Formation shale; the shales are of similar age ($\sim 50 \times 10^6$ yr) but the Messel appears to have experienced milder thermal conditions ($\sim 40$°C, cf. 90–125°C for the Green River shale—BRADLEY, 1970). Both shales are derived from shallow lacustrine paleoenvironments with sub-tropical climates, but the Green River Formation was deposited by an extensive lake, contrasting with the much smaller area of the Messel environment which comprised a series of small swamp-lakes linked by slow-moving fluvial systems. The biological input to the Green River Formation shale is thought to have been mainly algae (Xanthophyceae, Chloropyceae, Cyanophyceae) together with water-borne or wind-blown pollens and spores (BRADLEY, 1970). Available data for the Messel shale (SITTLER, 1968; SCHULER, 1971) show that pollens from Pteridophyta, Myricaceae, Fagaceae (Castaneoideae) and Cupressaceae predominated in the paleoenvironment.

The alkane:alkene ratios of the two shales (*ca.* 2:1 and 1:4 for Green River and Messel, respectively) may reflect the milder diagenetic/maturation history of the Messel shale. Tri- and tetraterpenoid alkanes are present in high relative abundance in both shales; however, the only major Messel tetraterpane is the acyclic lycopane (*ca.* 3·5 per cent of branched and cyclic alkanes), whereas the bicyclic perhydro-$\beta$-carotene (*ca.* 16 per cent of branched and cyclic alkanes) is the major tetraterpane of the Green River shale. The major Messel steranes are the 4-methyl compounds which are less abundant than the triterpanes present, whereas those of the Green River shale are the 4-desmethyl analogues which are more abundant than the triterpanes (BURLINGAME *et al.*, 1965; HILLS *et al.*, 1966; HENDERSON *et al.*, 1968a, b; ANDERSON *et al.*, 1969; GALLEGOS, 1971; ANDERS and ROBINSON, 1971). These carbon skeleton differences are also evident in the corresponding stanol fractions (MATTERN *et al.*, 1970; STEEL and HENDERSON, 1972).

Differences are also apparent in the pentacyclic triterpane fractions when the Messel components are compared with the triterpanes of the Green River shale isolated by several investigators (Table 3). It is difficult to compare the data from several investigators who have used different techniques to study the Green River shale triterpanes, but the comparison in Table 3 appears to be valid because of the overall similarities in the different gas chromatographic distributions. An abundant Green River component is gammacerane (14) but no pentacyclic triterpanes with all six-membered rings could be detected in the Messel shale. The most abundant Messel triterpanes are the hopane series (3a–e), with the 17$\alpha$H series (3a–d) present in lower concentrations. In the Green River shale the 17$\alpha$H compounds (3b–d) predominate.

Taken together, the differences in tetraterpane, sterane and triterpane skeletons in the two sediments must reflect differences in the organisms contributing the precursor polyterpenoids, and therefore differences in the paleoenvironments. This also suggests that identification of the complete suites of the structurally-specific alkanes in a range of sediments may provide a chemical method of classification and characterization of paleoenvironments. 4-Methylsterols, acyclic carotenes and hopane-type triterpenoid hydrocarbons are abundant components of certain prokaryotic organisms, and identical or structurally-related compounds are found in the Messel shale suggesting a significant microbial contribution to the sediment. The hopane-type skeletons may also be derived in part from the hydrocarbons of Pteridophytes (ferns). These may be inferred to be one of the predominant species in the Messel paleoenvironment from the available data on fossil plants and pollens (SITTLER, 1968; SCHULER, 1971), and such compounds are relatively abundant in present-day Pteridophytes.

The reversal of the hopane: 17$\alpha$H-hopane triterpane abundances in the two sediments may also reflect the milder diagenetic/maturation history of the Messel shale, as suggested by ENSMINGER *et al.* (in press).

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